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الجامعة الأردنيـــــة
كلية الدراسات العليا

التاريـــخ: / /

نموذج رقم (١٨)
اقرار والتزام بالمعايير الأخلاقية والأمانة العلمية وقوانين الجامعة الأردنية وأنظمتها وتعليماتها لطلبة الماجستير

أنا الطالب: اسراء وليد رجب سالم الرقم الجامعي: ٨٠٧١٥٧٨

تخصص: وقاية النبات الكليـــــــة: الزراعه

عنوان الرسالة: **EFFECT OF THREE OVICIDES ON THE REPRODUCTION OF A SUSCEPTIBLE STRAIN AND TWO FIELD STRAINS OF TWO SPOTTED SPIDER MITE, *Tetranychus urticae* KOCH (ACARI: TETRANYCHIDAE) FOR THREE GENERATIONS**

اعلن بأنني قد التزمت بقوانين الجامعة الأردنية وأنظمتها وتعليماتها وقراراتها السارية المفعول المتعلقة باعداد رسائل الماجستير عندما قمت شخصيا" باعداد رسالتي وذلك بما ينسجم مع الأمانة العلمية وكافة المعايير الأخلاقية المتعارف عليها في كتابة الرسائل العلمية. كما أنني أعلن بأن رسالتي هذه غير منقولة أو مستلة من رسائل أو كتب أو أبحاث أو أي منشورات علمية تم نشرها أو تخزينها في أي وسيلة اعلامية، وتأسيسا" على ما تقدم فانني أتحمل المسؤولية بأنواعها كافة فيما لو تبين غير ذلك بما فيه حق مجلس العمداء في الجامعة الأردنية بالغاء قرار منحي الدرجة العلمية التي حصلت عليها وسحب شهادة التخرج مني بعد صدورها دون أن يكون لي أي حق في التظلم أو الاعتراض أو الطعن بأي صورة كانت في القرار الصادر عن مجلس العمداء بهذا الصدد.

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الجامعة الأردنيـــة
كلية الدراسات العليا

التاريـــخ: / /

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تعتمد كلية الدراسات العليا
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EFFECT OF THREE OVICIDES ON THE REPRODUCTION OF A SUSCEPTIBLE STRAIN AND TWO FIELD STRAINS OF TWO SPOTTED SPIDER MITE, *Tetranychus urticae* KOCH (ACARI: TETRANYCHIDAE) FOR THREE GENERATIONS

By

Israa Waleid Rajab Salem.

Supervisor

Dr. Tawfiq M. Al-Antary, Prof.

This Thesis was Submitted in Partial Fulfillment of the Requirements for the Master's Degree of Plant Protection.

Faculty of Graduate Studies

University of Jordan

March 2011

COMMITTEE DECISION

This Thesis "Effect of Three Ovicides on the Reproduction of a Susceptible Strain and Two Field Strains of Two-spotted Spider Mite *Tetranychus urticae* KOCH (Acari: Tetranychidae For Three Generations" was successfully Defended and Approved on March ,2011

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EFFECT OF THREE OVICIDES ON THE REPRODUCTION OF A SUSCEPTIBLE STRAIN AND TWO FIELD STRAINS OF TWO SPOTTED SPIDER MITE, *Tetranychus urticae* KOCH (ACARI: TETRANYCHIDAE) FOR THREE GENERATIONS

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DEDICATION

To My Family

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Effect of Three Ovicides on the Reproduction of a Susceptible Strain and Two Field Strains of Two Spotted Spider mite, *(Tetranychus urticae* Koch) (Acari: Tetranychidae) for Three Generations

By

Israa Walied Rajab Salem

Supervisor
Dr. Tawfiq M. Al-Antary, Prof.

Abstract

Laboratory bioassays were conducted to evaluate the effects of chemical control compounds on the fecundity, hatchability and longevity of two-spotted spider mite *Tetranychus urticae* after treatment of egg stage of two spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) as a Syrian ,Al-Baq'a, Deir-Alla populations in three successive generations against some acaricides which selected from different groups. The response of the mite populations against pesticides estimated by calculating the median lethal concentration (LC_{50}). According to LC_{50} values, toxicity of the acaricides to the eggs of two spotted spider mites showed that etoxazole was the highest toxic than clofentezine and hexythiazox. Deir-Alla field population was more sensitive than Al-Baq'a field population against etoxazole and clofentezine, but it was more resistant than Al-Baq'a field population against hexythiazox. According to LC_{50} value in ppm of field population divided by LC_{50} value in ppm of Syria population, lower resistance factor indicated that the acaricide was more toxic, the population with lower resistance ; hexythiazox was less toxic than etoxazole and Clofentezine. Deir-Alla field population was higher in résistance than Al-Baq'a field population . RF value was very high for the two field populations against hexythiazox. For this reason hexythiazox is suggested to be unsuitable for use as a chemical pesticide for two spotted spider mite control program and to avoid its use for two years at least . During this period population was lead to remove the gene responsible for hexythiazox and then to produce a population more susceptible to hexythiazox.

1. Introduction:

The two-spotted spider mite (TSSM), *Tetranychus urticae* Koch (Acari: Tetranychidae), is one of the most important arthropod pest species worldwide. Among spider mites, *T. urticae* is the most polyphagous species. A recent checklist of host plants included about 1,200 species (Zhang, 2003), including fruits, cotton, vegetables and ornamentals (Mark*,* 2005).

The life cycle of the tetranychids consists of the egg, larva, protonymph, deutonymph and adult stages. Development occurs between 12 and 40°C. Developmental time from egg to adult decreases with an increase in temperature, and it takes less than a week at optimal temperatures (30-32°C) for development (Zhang, 2003). Each female can lay over than ten eggs per day and each female produces over 100 eggs during two weeks at about 25°C (Zhang, 2003). It feeds on the different plant parts such as buds, leaves, and fruits. It may transmit plant diseases causing great damage to the economical crops (El Kady *et al.,* 2007).

Chemical control is the primary method used for the control of eggplants plant pests in Florida (El-Saiedy *et al.,* 2008). Control of *T. urticae* populations in field crops, and particularly in protected crops, relies on the use of acaricides. However, the ability of this species to develop resistance is a major cause of control failure (Stumpf *et al*., 2001; van Leeuwen *et al.,* 2008*)*. Recently, there is a tendency to combine acaricides in order to prevent multiple uses of single acaricides, and to minimize the development of acaricide resistance in spider mite populations (Akio *et al.,* 2000). Spider mite control in 1970’s and

1980's relied upon a number of acaricides, belong to organochlorines and organophosphates (Khajehali and Van Leeumen, 2009). This reliance on chemicals has generally caused mite resistance and public concerns on their high residues in products (Dagli and Tunc, 2001). Some acaricides, such as dicofol, cyhexatin and fenbutatin oxide, have thus been prohibited from mite control on vegetables, melons, fruits and tea (Dagli and Tunc, 2001), making it necessary to search for alternative chemicals for spider mites control. Few insecticides are effective for spider mites and many even aggravate problems. Furthermore, strains of spider mites resistant to miticides frequently develop and make control difficult. Because of most miticides do not affect eggs, a repeated application at an approximately 10 to 14 day interval is usually needed for control (Sáenz-de-Cabezónand and Frank, 2006).

The use of insect growth regulators is an interesting approach. Benzoylphenil urea inhibits chitin synthesis in a wide range of insect groups, resulting in abortive moulting. They act mainly as larvicides and ovicides (Sáenz-de-Cabezónand and Frank, 2006). Effects on adults' fecundity, fertility, and longevity have also been reported (Sáenz-de-Cabezón *et al.,* 2002). Acaricidal activity has been also mentioned by several authors for various spider mites, including *T. urticae*, but little information is available about their effects on this kind of pests. Other pesticides used for controlling spider mites are available, they act as ovicidal, but also little information about their effect. In Jordan Nazer (1982), studied the toxicity of different acaricides against *T. urticae* population collected from Jordan Valley. Results obtained in this study revealed that amitraz was the most effective acaricide followed by dicofol and fenpropathrin. Nazer (1985), studied the toxicity of different acaricides against *T. urticae* population collected from Baqa region. He found that

cyhexatin and fenpropathrin acaricides were the most effective acaricides followed by dicofol.

Compounds used to combat plant damaging mite especially spider mites often destroyed the natural enemies of the mites without eradication the mite itself. Since mites develop resistance to the chemicals, their control has become a problem. To reduce mite resistance problem, specific pesticides should be used against mites only, which have different modes of action, so that they can be used in rotation to prevent resistance from development. It is needed to determine the effectiveness of the acaricides on different mite stages starting with egg stage for three successive generation (Ako *et al,* 2006).

The objectives of this study are:

1. Determining the efficacy of three acaricides on the hatch rate of three different populations collected from different locations.
2. Effect of the acaricides toxicity on longevity and fecundity of survived mites for three generations.

2. Literature Review:

2.1. Life cycle of two-spotted spider mite

Teranychid mite develops through egg, larva protonymph, deutonymph, and adult stage. The nymphal and adult stage are initiated during intervening periods of inactivity called protochrysalis, deutochrysalis and teliochrysalis . During these periods the mite anchors itself to a leaf or to its webbing. Females lay eggs on the undersides of leaves. The spherical egg is about 0.14 mm in diameter. The newly deposited egg is clear, but turns opaque and glassy as incubation progresses. Just before hatching, the egg is straw colored and the carmine "eyespots" of the embryo become visible. The larva (Pate1) has 3 pairs of legs. At the time of hatching, it is colorless, except for the carmine eye spots. After feeding, its color changes to pale green, brownish green or very dark green and two dark spots appear in the mid-portion of the body. At the end of the feeding stage, the larva attaches to the leaf, becomes quiescent (nymphochrysalis), and is later transformed into a protonymph. The protonymph has 4 pairs of legs and somewhat larger than the larva. Its color is usually pale green to dark green and the two spots are larger and more pronounced than in the larva. At the end of the feeding stage, the protonymph attaches to the leaf, enters the quiescent stage (deutochrysalis), and is later transformed into a deutonymph (Plate 1) (Jeppson *et. al.,* 1975).

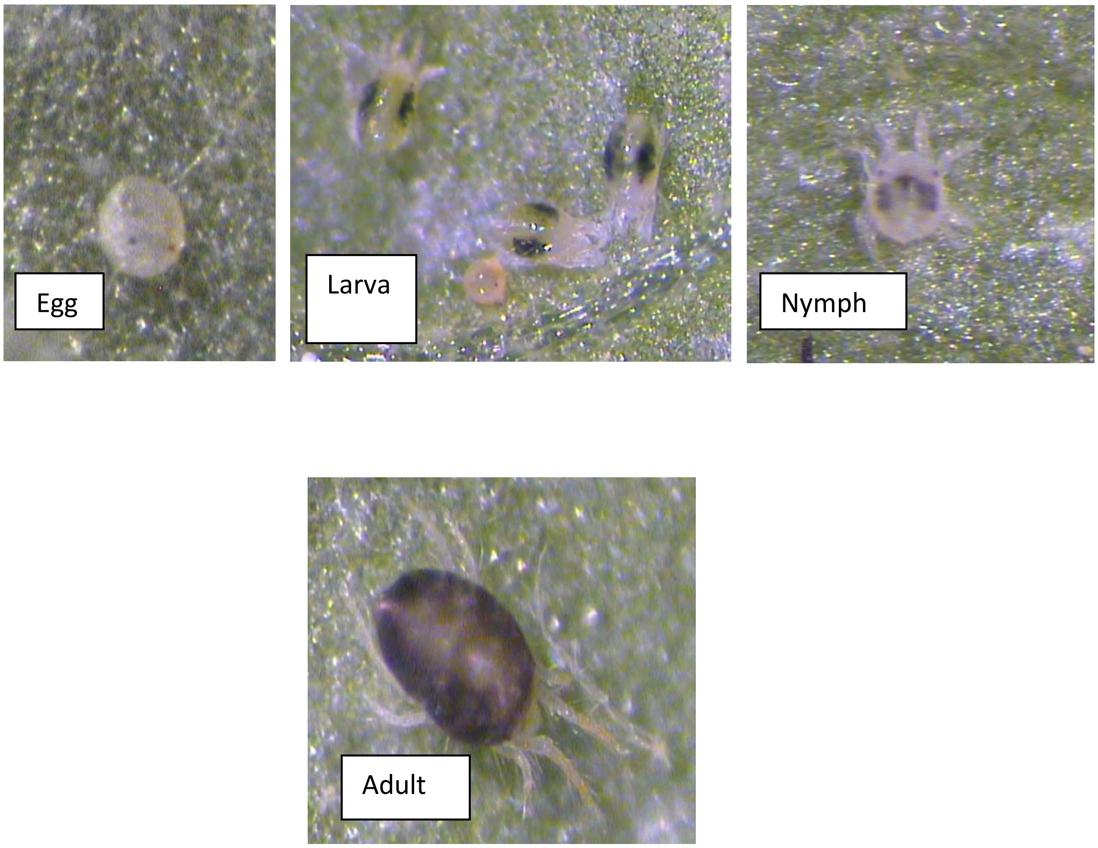


Plate (1): Lifecycle of *Tetranychus urticae* as shown by dissecting binocular microscope (magnification 80X).

The deutonymph is generally larger than the protonymph, although similar in color pattern. At this stage, males can usually be distinguished from the females because of the smaller size and wedge-shaped posterior of the former (Zhang, 2003). Following cessation of feeding, the deutonymph attaches to the leaf and becomes quiescent

(teliochrysalis). The adult eventually emerges from the teliochrysalis (Jeppson *et. al.,* 1975).

Developmental time of the two spotted spider mite will generally vary with conditions such as temperature, humidity, host plant, leaf age, etc. However, temperature is the most important factor that influences the rate at which mites develop. The lower threshold for development is about 12°C, whereas maximum upper limit to the development is about 40°C (Zhang, 2003).

2.2Biology of two-spotted spider mite

In a given colony of two spotted spider mites, both adult males and females can usually be found. (Zhang, 2003) provided an account of the characteristics of males and females. The body of female is oval-shaped and rounded posteriorly. The female adult is about 0.5 mm long and has eight short legs. Its color varies from light yellow or green to dark green, straw color, brown, black and various shades of orange. The male is much smaller and is considerable more active. The body is narrow and distinctly pointed posterioly. The color of male varies from pale to dark green, brownish, orange. However, females are normally about three times more abundant than males.

There are two large black spots one on either side of the body, hence the common name. However, there can be considerable variation in the expression of this particular character (Zhang, 2003).

Adult males can be found in close association with quiescent female deutonymph. Evidence indicates that the quiescent female deutonymph releases a sex pheromone which attracts the male and keeps him in close proximity (Keiko, 2008). The male usually remains

in the immediate vicinity of the quiescent deutonymph and mates with the emergent female. When more than one male attempts to "guard" a developing female, fighting among the males often occurs; usually, larger males win these encounters (Keiko, 2008). Such fights involve pushing and grappling with the forelegs, jousting with the mouth parts and entangling the opponent with silk. The life span of the adult female is divided into the pre-ovipositional period and the ovipositional period, the former being the time between emergence from the teliochrysalis to the deposition of the first egg. Apparently, the pre-ovipositional period (9% of the time required to develop from egg to egg) can last less than 0.5 day and as long as 3 days depending on temperature. The period during which eggs are deposited (ovipositional period) can last from 10 days at 35°C to 40 days at 15°C (Olivier, 1999). An individual female can deposit over 100 eggs in its lifetime (Zhang, 2003). The total number of eggs laid / female and the eggs laid / female / day vary with age, temperature, species of host plant, relative humidity, nutrition of host plant, exposure to pesticides, etc. (Thomas and Denmark, 2009). Temperature and age of the female are especially important determinants of egg production. However, Zhang (2003) determined that fecundity was affected very little at temperatures between 20-35°C. In his study, peak oviposition, (161 eggs / female) occurred at a temperature of 25°C; with the maximum rate (12 eggs / female/day at 25°C) occurring 2 days after the first eggs are laid. The effect of temperature is particularly evident in greenhouses, where spider mite populations often develop rapidly soon after the onset of summer temperatures.

Sex determination in two spotted spider mites is arrhenotokous. That is, females develop from fertilized eggs and have the normal two sets of chromosomes (diploid). On the other hand, males develop from unfertilized eggs and have only one set of

chromosomes (haploid). Unmated females give rise only to males. Mated females can produce either female or male progeny. According to some authors a single mating will suffice to provide a female with enough sperm to produce diploid eggs for its entire ovipositional period.

The potential for development of genetic resistance to insecticides and miticides in the two spotted spider mite is greatly enhanced by this method of reproduction. Because of the high reproductive rate and fast generation time and the intense selection pressure brought on by chemical control of this pest in the greenhouse. Resistance may develop in a comparatively short time (Abderrahman and Miodrag, 2007).

2.3. Damages and economic importance caused by two spotted spider mite

Two spotted spider mites feed on many species of plants. They are major pests of vegetables, ornamentals, fruit trees, hops, cotton, and strawberries (van den Boom *et al.*, 2003). At present, it is safe to assume that most of the major spider mite problems in greenhouses will involve the two spotted spider mite. This mite has been reported infesting over 200 species of plants. The two spotted spider mite is also a serious pest in greenhouses as well as on field grown chrysanthemums. The mites generally feed underneath the leaves and cause graying of the leaves due to mesophyll collapse and yellowing. Necrotic spots occur in the advanced stages of leaf damage. Mite damage to the open flower causes a browning and withering of the petals that resembles spray burn.

The larva, protonymph, deutonymph, and adult feed mainly on the undersides of the leaves. When feeding, the body of the mite is tipped upward such that the 3rd and 4th pairs of legs are off the leaf surface and the mite is supported by the 1st and 2nd pairs of

legs (Zhang, 2003) . Feeding is accomplished in the following manner: a pair of needle-like stylets penetrates the plants' parenchyma cells, the contents of which are then drawn into the body of the mite by a "pharyngeal pump". Protonymphs and deutonymph spend about half their developmental times feeding and half in the resting or quiescent stage. The larvae spend slightly more time feeding than resting (Thomas and Denmark, 2009).

Damage to the plants is affected in several ways. First, feeding causes the destruction or disappearance of chloroplasts which then leads to basic physiological changes in the plant. Stomata closure can be a primary host-plant response, and in such cases, uptake of CO_2 decreases resulting in a marked reduction in transpiration and photosynthesis. These effects can occur at spider mite densities that are too low to cause visible damage. Reduction of photosynthetic area by spider mite feeding is permanent and can only be compensated for by production of new foliage. Methods have been developed to quantify the amount of feeding and therefore damage for both cucumber and tomato. It is likely that the mites actually inject phytotoxin substances into the plant when feeding. The stippling or speckling of the upper leaf surface, plus the webbing produced by protonymphs, deutonymph, and adults, leads to aesthetic injury, particularly in the case of ornamental plants (Hussey and Parr ,1963).

The factors which determine the abundance or density of spider mites have been discussed in considerable detail by several workers (Jeppson *et al.,* 1975). With respect of outbreaks of spider mites, particularly since World War II, there are two central "hypotheses" or tentative explanations to account for these events. The first is that the upsurge of spider mites is due to improved cultural practices, such as pruning, fertilization, and pesticide uses. For example, outbreaks of spider mites can be induced by certain

fertilization practices or by certain pesticides, regardless of natural enemies (Jeppson *et al.,* 1975; Ken and Bruce, 2009). Apparently, these cultural practices increase the nutritive value of the plant and thus enable greater reproductive activity on the part of the spider mites. The second explanation is simply that widespread use of broad-spectrum insecticides destroys or greatly hamper natural enemies of spider mites and thereby allow pest outbreaks to occur (Jeppson *et al.,* 1975; Kenand Bruce, 2009). Most of the pertinent information in the literature concerns the influence of pesticides on outbreaks of spider mites under field conditions. According to Ken and Bruce (2009) increases in abundance of two spotted spider mites have been observed following use of certain agricultural chemicals in many different crops. Although the causes of such increases in greenhouses have not been determined, it would be a sound practice to minimize the use of insecticides and miticides in the greenhouse since outbreaks of two spotted spider mites in the field are often correlated with pesticide usage.

Probably the most common scenario for outbreaks of two spotted spider mites in greenhouses is as follows: The spider mites are accidentally introduced into the greenhouse without any of their effective natural enemies; if host plants and physical factors are suitable, the population "explodes". Common sources of inoculums include infested plants carried into the greenhouse, spider mites which cling to the clothing of greenhouse workers and weeds growing outside the greenhouse. However, chemical controls used to control other pests can destroy natural enemies which have been introduced into the greenhouses and thus, engender serious outbreaks of the two spotted spider mites. Thus, insecticides, miticides, and fungicides should be used judiciously when natural enemies are present in

order to minimize unnecessary problems with two spotted spider mites(Jeppson *et al.,*1975).

2.4. Acaricides toxicity

A- Hexythiazox:

Chemical control is the primary method used for the control of plant pests (El-Saiedy *et al.,* 2008). Control of *T. urticae* populations in field crops, and particularly in protected crops, relies on the use of acaricides. Some contact acaricides can be used for the control of red spider mite and reduce their reproduction. The efficiency of these components in controlling spider mites depends on strain of the mite and the covering of the plant (Binns, 1969). In the second generation resistance strain was developed (Binns, 1969). Hexythiazox is selective miticide, which is active against, various phytophagous mites and has no or little effect on their natural enemies (Yamamoto *et. al.,* 1995). Nadimi *et al.* (2008) reported the effects of hexythiazox and different pesticides on *Phytoseiulus. persimilis* used in the biological control programs in glasshouses. *P. persimilis* exposed to dry residues of hexythiazox at half and quarter of the field rate suffered only 5.43 to18.44% mortality. This miticide interferences with mite growth and reproduction and does not show cross resistance to conventional miticides. Hexythiazox has been used for controlling mites. Hexythiazox compound has excellent ovicidal, larvicidal and nymphicidal actions. Although hexythiazox does not kill adult mites, eggs laid by treated females do not hatch. The effects of pesticides on *T. urticae* are being widely studied and its resistance to new products is frequently monitored (Castagnoli *et al.,* 2005). Failures of chemical control of *T. urticae* caused by resistance have been reported in several countries for compounds such as hexythiazox .Two-spotted spider mites have a history of rapidly developing

resistance to miticides when a miticide is repeatedly applied to the same population , development of the hexythiazox resistance, however, was recently reported in the several species of the mites (Nadimi *et al,* 2008).

B- Etoxazole:

Etoxazole is a relatively new acaricide it was introduce to Jordan in 2005. Therefore studies on the toxicity and its effect on the life biology of the TSSM could not be enough. Ochiai *et al.,*(2007) studied the toxicity of the etoxazole and other pesticides against adult , larva and egg stages of *T. urticae.* Etoxazole showed no activity against adults of *T. urticae*. However, etoxazole was highly effective in controlling the larval and egg stages of *T. urticae* mite strain. Etoxazole is effective against eggs, larva and nymphs stage of mite , but lacks any efficacy against male and female. However, sterilizing effect on the adult female by exhibiting significant transovarial ovicidal activity. Therefore, when applications of the Etoxazole are made, at recommended field rate, newly laid eggs from treated females do not hatch (Sáenz-de-Cabezón and Frank*,* 2006).

C-Clofentezine

Clofentezine was recently introduced in China and provides good efficiency against egg and larval stages of spider mites. Clofentezine treatments in the juvenile stage significantly delayed development of the mites. This delay increased with increasing dose but decreased as the mites developed (Stumpf *et al.,* 2001).

Several reports on demographic techniques regarding sub-lethal effects of acaricides on spider mites have been published so far (Jones and Parrella , 1984, Manal *et*

al., 2007), none of which considered clofentezine, a well-known ovicides and growth inhibitors. Maximum longevity was 29 days for both the untreated and treated females. During the first 11 days, the female survival was similar in both cohorts, contrary to the last 18 days, during which the untreated females survived in a larger number. During the first 5 days of reproduction, the untreated females produced somewhat less offspring than those females that had survived treatment as 'early' eggs. On the sixth day, the difference disappeared and the fertility was continuously higher in the control until the end of the reproduction period (Marcic , 2007). Efficacy against *Tetranychus.* spp. began to decline in the mid-1990s in parts of Northern China due to the intensive use of this acaricide, and resistance was documented in China ,Japan and Australia (Herron *et al.,*1993). Despite this resistance development, clofentezine will continue to be used in IPM mite programs in Northern-Chinese apple orchards, because of the unavailability or withdrawal of compounds based on regulatory decisions, as well as delays in the registration of new acaricides. To enhance its effective use, therefore, it is important to determine its lethal and sub-lethal effects at the population level of the mites (Dingxu et. al., 2006).

The current trend in pesticide development is to release less broad-spectrum and more specific pesticides, in order to avoid negative effects on non-target organisms, while still providing control of the target pest. Consequently, growers can encounter a range of insect and mite pests simultaneously therefore, mixtures of different pesticides are used to manage the diversity of arthropod pests present in a crop. Although there are concerns associated with pesticide mixtures such as enhanced resistance to one or more pesticides, phytotoxicity, and pesticide incompatibility and antagonism (Warnock and Cloyd ,2005).(Sáenz-de-Cabezónand and Frank (2006).observed an ovicidal activity of some

insect growth regulators pesticides against *T. urticae* as a consequence of a transovarial transmission of the compound. Also, they observed a substantial reduction in egg viability when treating *T. urticae* deutonymph females with insect growth regulators pesticides. Other kinds of insect growth regulators also caused a reduction in the percentage of eggs hatched when the compound was applied to *T. urticae* adult females.

3. Material and Methods:

3.1. Bean plant production

Seeds of bean *Phaseolus vulgaris* L. *cv* cultivar (Bronco) were continuously planted in seed trays until it reached 5-6 true leaves. The seedlings were transplanted in plastic pots containing peat moss. All plants were kept in the greenhouse for mite rearing. The temperature in the green house ranged between (27-38°C) with an average of 32°C during the period of rearing. Plants were irrigated when needed. Pesticides were not applied on these plants except later on for testing.

3.2. Population of two-spotted spider mite used

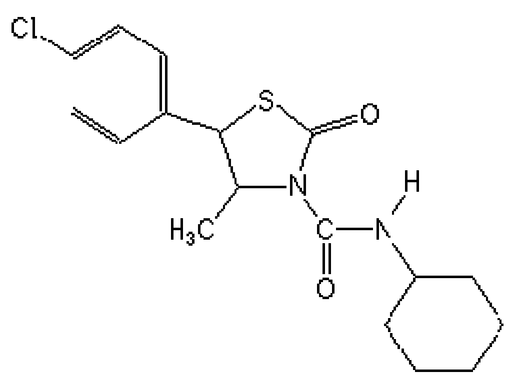
The experimental were conducted on the three different mite populations from different locations. The first was obtained from Lattakia Center for Rearing and Production of Biological Agent (LCRPBA) in Syria .This population was reared in (LCRPBA) for five year without application of acaricides. Syrian population maintained in green house , Faculty of Agriculture, University of Jordan, starting in June , 2009. Other population of *T. urticae* was brought from cucumber plants from Deir-Alla and Al- Baq'a. Mites of all population were reared continuously on potted kidney bean plants, under conditions of the greenhouse (27-38°C.) with averaged (32°C.) and 50–70% R.H. Some kidney bean plants were infested with *T. urticae*. The plants were renewed gradually and when necessary and from time to time infested leaves with *T. urticae* susceptible population were distributed on the plants. This culture was kept during the whole period of study and used for re-infestation of

the newly established culture in the green house. Each population found inside different location of the green house inside sterilized pinch.

3.3.Acaricides used

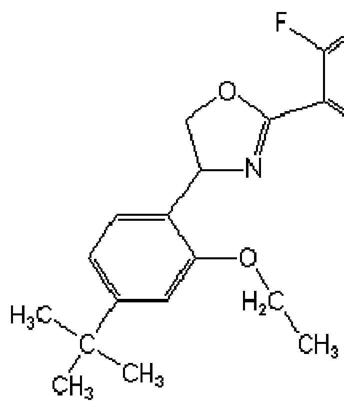
Among many acaricides used in Jordan three of these were selected to test in this trial .A fresh stock solution of each pesticide was prepared in the tap water on each test day. All further dilutions were prepared from the stock solution. Tap water was used as control. The pesticides used in this study as commercial formulations were :

Hexythiazox : Nissorun 10% WP,chemical group is carboxamide ,non-systemic with contact and stomach action, empirical formula: $C_{17}H_{21}ClN_2O_2S$, produced by Nippon Soda Co. LTD., Japan .Recommended application rate is0.5g / L of water and its structure and chemical name as follows.



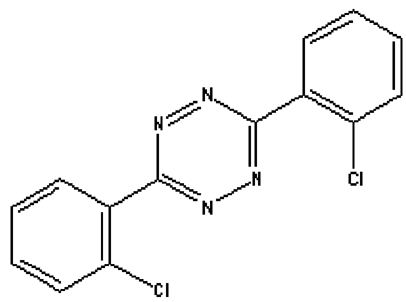
(4*RS*,5*RS*)-5-(4-chlorophenyl)-*N*-cyclohexyl-4-methyl-2-oxo-1,3-thiazolidine-3-carboxamide, (Tomline , 2005).

Etoxazole: Baroque 10% (SC), chemical group: Diphenyl oxazoline, non-systemic with contact action, empirical formula: $C_{21}H_{23}F_2No_2$,produced by Valent, Yasshima Chemical Industry, Sumitomo Chemical, with recommended rate of application 0.5 cm^3/L of water and its structure and chemical name as follows:



, (*RS*)-5-tert-butyl-2-[2-(2,6-difluorophenyl)-4,5-dihydro-1,3-oxazol-4-yl]phenetole (Tomline , 2005).

Clofentezine: Apollo 50% (SC), chemical group: Tetrazine, specific acaricide with contact action and long residual activity .Inhibits embryo development, empirical formula: $C_{14}H_8Cl_2N_4$, produced by Irvita Plant Protection N. V. Canada, with recommended rate of application 0.5cm^3 / L of water and its structure and chemical name as fallows as :



3,6-bis(2-chlorophenyl)-1,2,4,5-tetrazine (Tomline , 2005) .

3.4. Laboratory assessments of acaricides toxicity

Preliminary tests including the control were done for each acaricides to determine the concentrations that would be used in this study. Kidney bean plants leaves, *Phaseolus vulgaris* were placed on wet cotton in a 9cm Petri dish. 10–20 gravid adult female mites were placed on the leaf discs before spraying and permitted to lay eggs for 24 h, and then were removed. Numbers of eggs on leaf were 30-50 eggs. Immediately after removal of the adults and before eggs hatching, the leaves were treated with the acaricides use.

Five concentrations of each acaricides were sprayed by a small hand sprayer (1liter in size) and left to dry for two minutes. Tap water was used as a control for each pesticide for each concentration. Four replicates for each concentration were used. Mortality was assessed after 6-8 days. Eggs on treated leaf were considered dead if they did not hatch. The SPSS computer program revision 13 (S. Hardware.) was used for data analysis to estimate LC_{50}, LC_{90} values. The acaricides concentration (ppm) ranged:

Table (1): The hexythiazox concentrations (ppm) used to determine LC_{50} value for different populations of two spotted spider mite.

Syrian Population	Baq'a Population	Deir-Alla Population
0.02 ppm	275 ppm	200 ppm
0.07 ppm	300 ppm	300 ppm
0.09 ppm	325 ppm	400 ppm
0.13 ppm	350 ppm	500 ppm
0.18 ppm	375 ppm	600 ppm

Table (2): The etoxazole concentrations (ppm) used to determine LC_{50} value for different populations of two spotted spider mite.

Syrian Population	Baq'a Population	Deir-Alla Population
0.02 ppm	0.15 ppm	0.28 ppm
0.05 ppm	0.30 ppm	0.19 ppm
0.09 ppm	0.45 ppm	0.17 ppm
0.11 ppm	0.60 ppm	0.11 ppm
0.15 ppm	0.75 ppm	0.05 ppm

Table (3): The clofentezine acaricide concentrations (ppm) used to determine LC_{50} value for different populations of two spotted spider mite.

Syrian Population	Baq'a Population	Deir-Alla Population
5 ppm	10 ppm	10 ppm
10 ppm	20 ppm	20 ppm
15 ppm	30 ppm	30 ppm
20 ppm	40 ppm	40 ppm
25 ppm	50 ppm	50 ppm

The temperature ranged in the laboratory 22 - 33.6° C with average (27° C) during the period of pesticide toxicity testing, while the relative humidity was 43%-58.3%. The experiment was conducted under natural room light during the day and under florescent light during night.

3.5. Effect of acaricides on hatchability of two-spotted spider mite for three generations.

To provide eggs to this test, disks of bean leaves were placed in Petri dishes lined with water-saturated cotton wool. (Ten to twenty female mites were introduced on the lower surface of the disks before spraying and permitted to lay eggs for 24 h, then they were removed). Four replicates were used (30-50 eggs/leaf). Immediately after removal of the adults, the leaf was sprayed with LC_{50} acaricides concentrations. The Petri dishes were dried for at least two minutes. After drying, the Petri dishes were placed at laboratory room conditions, under natural room light during the day and under florescent light during night..

Average temperature was 26.4 ° C (ranged: 22.5-30.5° C) for Syrian population and 24.1° C (ranged: 25-30° C) for two field population with average relative humidity about 57.3% for Syrian population and 58.3% for two field populations.

All Petri dishes were examined daily for seven successive days. Hatchability (% hatching) was recorded after six to eight days by counting the numbers of hatched eggs on the leaf. Control individuals were sprayed with tap water in four replications .All acaricides were separately examined for three generation of survived mites to determine acaricide toxicity on the TSSM egg hatchability of the three populations after treatment with its LC_{50}.

3.6. Effect of acaricides on fecundity of two-spotted spider mite for three generations.

The offspring of the egg treated in the peruses trial were transferred. A group of five pre-ovipositing females' adult age, were placed on each leaf and allowed to oviposit on it under natural room light during the day and under natural room light during the day. Average temperature was 26. 6° C (ranged: 25-29° C) for Syrian population and 26.6° C (ranged: 25-31.5 ° C) for two field population with relative humidity about 55.3% for Syrian population and 50.1% for two field populations. To avoid food shortage, the bean leaf tissues were replaced with fresh leaves 3 days after introduction. The numbers of eggs oviposited were counted at 3-days intervals over a 15-days period. Four replications were performed for each acaricide. Fecundity was expressed as the mean number of oviposited eggs by one female over one day. All acaricides were examined for three generations of survived mites to determine acaricides toxicity separately on the fecundity of TSSM adult of the three populations after treatment with its LC_{50}.

3.7 Effectof the three acaricides on longevity of two-spotted spider mite for three generations.

The offspring of the egg treated in the peruses trial were transferred. A group of seven to ten adult females age were transferred with a brush to bean leaves kept on moist cotton wool pads in Petri dishes. The experiment was conducted under natural room light during the day and under florescent light during night. Average temperature about 27.2 ° C (ranged : 22.5- 32° C) for Syrian population and 28.4° C (ranged: 25-32 ° C) for two field population with relative humidity about 48.6 % for Syrian population and 43.2 % for two field populations. The number of alive females was monitored daily until their death. To avoid food shortage, the bean leaf tissues were replaced with fresh leaves 3 days after introduction. Four replications were performed for each acaricide. All acaricides were examined for three generations of survived mites to determine acaricides toxicity separately on the longevity of TSSM adult of the three populations after treatment with its LC_{50}.

4. Results

Data analyses for the experiment of laboratory assessments of pesticides toxicity for the three population of TSSM, has been constructed in Tables (1, 2 and 3) to facilitate comparison between different acaricides used against the egg stage of TSSM.

Goodness of line fitting was checked by Chi-square test X^2 .The value of X^2 at 0.05 level of probability equals to 2.227, 2.760 ,1.503 at 3 degrees of freedom (df) for Baq'a field population , Deir-Alla field population and Syrian population, respectively, for etoxazole, and 0.933,1.75 ,0.292 at 3 df for Baq'a field population, Deir-Alla field population and Syria population, respectively, for clofentezine, and 2.83, 2.25, 4.94 at 3 df for Baq'a field population, Deir-Alla field population and Syria population, respectively, for hexythiazox.

4.1. Toxicity of hexythiazox to two-spotted spider mite

Comparison between the LC_{50} of the hexythiazox acaricide for the three population of TSSM (Table 4) showed that , the lowest LC_{50} is for Syria population (0.10 ppm), followed by AL-Baq'a field population (331ppm) then Deir-Alla field population (370ppm). LC_{50} for Baq'a field population and Deir-Alla field population were not significantly different (95%CL overlap), while LC_{50} for the two field population with LC_{50} for Syrian population were significantly different .Y value for each line estimated by probit regression was equal to zero when LC_{50} (x) was converted to log base 10.

Table (4): Comparative (LC_{50}) and (LC_{90}) of hexythiazox acaricide tested on various population of *T. urticae* in the laboratory

Populations	$LC_{50\ ppm}$*	95% CL[1]	LC90	95% CL	L.E.P.R [2]	Slope ± SE	R.F***
Syrian	0.10a	0.07-0.12	0.24 a	0.17 - 0.61	Y= 3.60+3.62X	3.62 ± 1.01	-
Baq'a	331b	321.9.5-340	386.17b	370.3-415.11	Y= -48.4+19.2X	19.2 ± 7.58	3310
Deir-Alla	370bc	317-422	900 c	738.2-1248	Y= -8.55+3.33X	3.33 ± 1.26	3700

1 = 95% confidence limits for LC_{50} in ppm.

2=L.E.P.R. Line estimated by Probit regression.

*=LC_{50} values having different letters are significantly different (95% did not overlap).

**=LC_{90} value in ppm having different letters are significantly different (95% did not overlap). .

***=LC_{50} value in ppm of field population divided by LC_{50} value in ppm of Syrian population, lower resistance factor indicated that the pesticides were more toxic, the populations with lower resistance

4.2. Toxicity etoxazole on two-spotted spider mite

Comparison between the LC_{50} of the etoxazole acaricide for the three populations (Table 5) showed that, the Syrian population had lowest LC_{50} (0.08ppm) than Deir-Alla field population (0.15ppm) followed by Baq'a field population (0.43ppm). LC_{50} for Syria population was significantly different (95% CL_{50} not overlap), while LC_{50} for Al-Baqa field population and Deir- alla field population were significantly different (95%CL overlap) when compared the two field population. Y value for each line estimated by probit regression was equal to zero when LC_{50} (X) was converted to log base 10.

Table (5): Comparative (LC_{50}) and (LC_{90}) of etoxazole acaricide tested on various populations of *T. urticae* in the laboratory.

Populations	LC_{50} ppm*	95%CL[1]	LC_{90} ppm**	95%CL	L.E.P.R [2]	Slope ± SE	R.F***
Syrian	0.08a	0.067 - 0.098	0.23 a	0.17 - 0.44	Y= 3.05+2.82X	2.82 ± 0.59	-
Baq'a	0.43c	0.37-0. 51	1.07c	0.83 - 1.86	Y= 1.17+3.25X	3.25 ± 0.22	5.375
Deir-Alla	0.15b	0.12 - 0.16	0.35b	0.28 -0.57	Y= 2.72+3.25X	3.25 ± 0.47	1.875

1 = 95% confidence limits for LC_{50} in ppm.

2=L.E.P.R. Line estimated by probit regression.

*=LC_{50} values having different letters are significantly different (95% did not overlap).

**=LC_{90} value in ppm having different letters are significantly different (95% did not overlap).

***=LC_{50} value in ppm of field population divided by LC_{50} value in ppm of Syrian population, lower resistance factor indicated that the acaricides were more toxic, the population with lower resistance .

4.3. Toxicity of clofentezine on two-spotted spider mite

Comparison between the LC_{50} of clofentezine acaricide for the three different populations (Table 6) showed that, the lowest LC_{50} was for the Syrian population (16.56 ppm) followed by Deir-Alla field population (27.4 ppm), then Baq'a field population (29.7 ppm). There were significant differences between LC_{50} of Syria population and Baq'a field population (LC_{50} not overlap) and with significant differences between Syria population and Deir-Alla field population (LC_{50} not overlap). There were no significant difference between the two field populations; Baq'a field population and Deir-Alla field population. Y value for each line estimated by probit regression was equal to zero when LC_{50} (X) was converted to log base 10.

Table (6): Comparative (LC_{50}) and (LC90) of clofentezine acaricide tested on various populations of *T. urticae* in the laboratory.

Populations	LC_{50} ppm*	95%CL [1]	LC_{90} ppm**	95%CL	L.E.P.R [2]	Slope ± SE	R.F***
Syrian	16.56a	13.9 - 20.2	48.4a	34.2 - 100.6	Y= -3.35+1.19X	1.19 ± 0.64	-
Baq'a	29.7c	25.08 - 34.8	76.7c	76.8 – 58.3	Y= -4.58+3.11X	3.11 ± 0.56	1.76
Deir-Alla	27.4bc	22.9-32.2	73.7bc	56.2- 123.2	Y= -4.30+2.98X	2.98 ± 0.77	1.65

1 = 95% confidence limits for LC_{50} in ppm.

2=L.E.P.R. Line estimated by Probit regression.

*=LC_{50} values having different letters are significantly different (95% did not overlap).

**=LC_{90} value in ppm having different letters are significantly different (95% did not overlap).

***=LC_{50} value in ppm of field population divided by LC_{50} value in ppm of Syrian population, lower resistance factor indicates that the acaricide were more toxic, the population with lower resistance

4.4. Effects of the three acaricides on the egg hatchability, adult longevity and fecundity of the two- spotted spider mite for three generations.

This experiment was carried out to determine the toxic effect of the tested compounds at LC_{50} level on the eggs hatchability, fecundity and adult longevity of different populations of the *T. urticae*. Result of hatchability, fecundity and adult longevity were analyzed by paired t-test in case of egg hatchability and ANOVA in case of longevity and fecundity. The comparison between the effect of different acaricides on the egg hatchability , fecundity and adult longevity of different population shown in Tables 7,8,9,10 and 11.

4.4.1. Effect of the three acaricides on the egg hatchability.

After 8 days of the eggs exposure to the LC_{50} values concentrations of different acaricides , the percentage of the eggs that survived after hexythiazox treatment were 60.50% for generation one **,** 71.75% for generation two,74% for generation three ,58.3% for generation one ,63.50% for generation two , 67.75% for generation three , respectively for Deir-Alla field population and Baq'a field population and 42.35%(G1), 35.625% (G2) and 29.37% (G3) for Syrian population (Table 7) .

The percentage of the egg hatching after 8 days of etoxazole was 42.62% for (G1), 35.62% for (G2) and 29.62 % for (G3) for Deir-Alla field population . The percentage of the egg hatching after 8 days of etoxazole were 54.4% , 45.68% for (G1) **,** 42.4% ,35.62% for(G2) and 25%, 22.50% for (G3) for Baq'a field population and Syrian population (Table 8). The percentage of Syrian population egg hatching with clofentezine egg treatment were 42.55 **%** for (G1) **,** 30 % for (G2) 26.66%, for (G3) .The results for

Deir- alla field population was54.94 for **%**(G1)**,** 48.88 **%** for (G2) , 42.42 % for (G3) and 48.75**%** for(G1)**,** 42.42**%** for (G2),36.49**%** for **(**G3) for Baq'a field population(Table 9).

Table (7): Effect of hexythiazox on % of egg hatchability of the different populations of the two spotted spider mite after the treatment with its LC_{50} , for three generations under laboratory conditions.

Populations	% Egg hatching ±SE					
	First generation		Second generation		Third generation	
	control	Treated with hexythiazox	control	Treated with hexythiazox	control	Treated with hexythiazox
Syrian	96.81±3.045a	42.35±2.39b	97.29±1.402a	35.625±1.173b	95.11±2.12a	29.37±2.196b
Baq'a	96.88±2.041a	58.3±4.26b	97±0.123a	63.5±1.19b	95.98± 1.089a	67.75±1.49b
Deir-Alla	95.50± 1.32a	60.500±1.32b	98.25±1.18a	71.75±1.75b	96.34±0.045a	74.00±1.19b

Means within the same row in the same generation that do not share the same letter are significantly different at 5% level according to paired t- test.

Table (8): Effect of etoxazole on % of egg hatchability of the different populations of the two spotted spider mite after the treatment with its LC_{50}, for three generations under laboratory conditions.

% Egg hatching ±SE						
Populations	First generation		Second generation		Third generation	
	control	Treated with etoxazole	control	Treated with etoxazole	control	Treated with etoxazole
Syrian	96.50± 0.523a	45.687±1.23b	97.29±1.402a	35.625±1.173b	96.25±1.52a	22.500±2.02b
Baq'a	96.27±1.045a	54.37±1.19b	97.55±1.20a	42.35±0.39b	95.62±0.197a	25±1.023b
Deir-Alla	96. 47± 0.033a	42.62±2.57b	96.24±1.423a	35.62±2.37b	96.34±0.045a	29.62±1.19b

Means within the same row in the same generation that do not share the same letter are significantly different at 5% level according to paired t-test.

Table (9): Effect of clofentezine on % of egg hatchability of the different populations of the two spotted spider mite after the treatment with its LC_{50} , for three generations under laboratory conditions.

Populations	% Egg hatching ±SE					
	First generation		Second generation		Third generation	
	control	Treated with clofentezine	control	Treated with clofentezine	control	Treated with clofentezine
Syrian	96.15±1.38a	42.55±1.616b	96.25±2.42a	30±2.347b	96±1.625a	26.66±1.175b
Baq'a	97.25±1.22a	48.75±0.72b	96.20±1.32a	42.42±2.45b	96.05±1.62a	36.49±0.748b
Deir-Alla	96.43±2.73a	54.94b ±0.33b	96.43±2.73a	48.88±2.091b	95.83±1.59a	42.42±2.46b

Means within the same row in the same generation that do not share the same letter are significantly different at 5% level according to paired t- test.

4.4.2. Effect of the three acaricides on the adult longevity.

The adult longevity for three generation of the three populations of *T. ureticae* treated with hexythiazox, etoxazole and clofentezine are shown in (Table7). The adult longevities were 33.97 for G1 , 32.82 for G2, 32.75 for G3 and 33.60 for G1 , 32.65 for G2, 32.97 for G3 and 33.70 for G1, 32.70 for G2, 32.45 for G3 for the three generation of Syria population ,Deir-Alla field population and Baq'a field population , respectively, that treated with LC_{50} value concentration of hexythiazox compound. However, longevity significantly decreased in the 2nd and 3rd generations in all populations compared with first generation . The effect of the etoxazole compound on the adult longevity of three generation of two spotted spider mite were 32.75 for G1, 33.17 for G2, 32.22 for G3 for the Syrian population and 33.50 for G1, 33.25 for G2, 32.50 for G3 for Deir-Alla field population and 33.67 for G1 , 33.55 for G2, 32.72 for G3 for Baq'a field population . However , longevity did not differ significantly in the three generations in all populations. The effect of the clofentezine compound on the adult longevity of three generation of two spotted spider mite were 32.075 for G1, 23.65 for G2, 16.20 for G3 for Syrian population and 32.52 for G1, 27.27 for G2, 14.82 for G3 for Deir-Alla field population and 31.100 for G1, 27.80 for G2, 13.13 for G3 for Baq'a field population . However, longevity was significantly different in the 2nd and 3rd generations compared with first generation in all population (Table 10) .

Table (10) : Effect of the three acaricides on the longevity of the two – spotted spider mite adults of the three populations after treatment with its LC_{50} , for three generations under laboratory conditions.

Populations	Longevity in days ± SE								
	Treated with hexythiazox			Treated with etoxazole			Treated with clofentezine		
	Generation			Generation			Generation		
	1	2	3	1	2	3	1	2	3
Syrian	33.97± 0.16a	32.82±0.19b	32.75±0.22b	32.75±0.30a	33.17± 0.21a	32.22±1.02a	32.075±0.23a	23.65± 0.06b	16.20 ± 0.18c
Baq'a	33.70±0.31 a	32.70±0.24b	32.45±2.34b	33.67±0.10a	33.55±0.17a	32.72±0.14a	31.10 ±0.48a	27.80±0.40b	13.13±0.46c
Deir-Alla	33.60±0.28 a	32.65±0.15b	32.97±0.27ab	33.50±0.20a	33.25±0.06a	32.50±0.26a	32.52±0.06a	27.27±0.21b	14.82±0.72c

Means within the same row in the same acaricide do not share the same letter are significantly different at 5% level according Duncan's multiple range test.

4.4.3. Effect of the three acaricides on the fecundity

The adult fecundity for the three generations of different population of *T. urticae* under the impact of three different acaricides during 15 days is presented in Table (8). The number of eggs laid at day 15 from adult stage of the Syrian population, Deir-Alla and Baq'a field populations treated with hexythiazox 64 , 75 and 74.75 for G1 and 30.75 , 78.75and 79.50 for G2 and 86.75 , 78.50, 82.25 for G3 and 128 , 82.75 and 78.75 for G4 respectively (Table 11). The number of the egg production by *T. urticae* adult with etoxazole treatment at 15 days were 61 for G1, 60.25 for G2, 63.75 for G3 and 68 for G4 for Syrian population and 61.75 for G1 , 66.50 for G2, 69.25 for G3 and 70 for G 4 of 15 days for Deir-Alla field population and 64 for G1 , 63.75 for G2, 65 for G3 and 68.25 for G4 for 69.25 for Baq'a field population treated with etoxazole (Table 11) . The number of the egg production by *T. urticae* adult for the three generation with clofentezine treatment at 15 days were 57.75 for G1, 48.50 for G2, 35.25 for G3 and 38 for G4 for Syrian population and 72.50 for G1, 56.25 for G2, 101.25 for G3and 139.75 for G4 for Deir-Alla field population and 66.25 for G1, 50.50 for G2, 79.50 for G3 and 132 for G4 at 15 days for Baq'a field population(Table 11) .

Table (11): Effect of the three acaricides on the fecundity of the two – spotted spider mite adults of the three populations after treatment with its LC_{50}, for three generations under laboratory condition.

Fecundity during 15day ± SE												
	Treated with hexythiazox				Treated with etoxazole				Treated with clofentezine			
Population	Generations				Generations				Generations			
	1	2	3	4	1	2	3	4	1	2	3	4
Syrian	64.00±1.63bc	30.75±1.70c	86.75±2.41b	128.00±4.2a	61.00±3.80a	60.25±1.37a	63.75±2.01a	68.00±5.61a	57.75±1.93a	48.50±2.64b	35.25±4.36c	38.00±1.47c
Baq'a	74.75±2.49a	79.50±4.04a	82.25±2.40a	78.75±1.37a	64.00±2.04a	63.75±2.59a	65.00±2.12a	68.25±1.25a	66.25±3.06bc	50.50±0.64c	79.50±2.12b	132±13.9a
Deir-Alla	75.00±1.82a	78.75±2.75a	78.503.56±a	82.75±2.95a	61.75±2.04a	66.50±2.56a	69.25±1.79a	70.00±2.97a	72.50±2.39bc	56.25±2.86c	101.25±2.32b	139.75±21.4a

Means within the same row in the same acaricide do not share the same letter are significantly different at 5% level according Duncan’s multiple range

5. Discussion

Resistance factors (RF) were calculated by dividing the LC_{50}-value of the field population by the LC_{50}-value of the Syrian population; Syrian population. LC_{50}-values of different pesticides were estimated by combining the results of three populations of *T. urticae* (laboratory and two field populations). This baseline LC_{50}-served as a reference for the calculation of resistance factors for hexythiazox, etoxazole and clofentezine in *T. urticae* populations. Results indicated that *T. urticae* field population was more resistant against hexythiazox when compared with the other pesticides; etoxazole and clofentezine. Generally hexythiazox was the least toxic against egg stage of *T. urticae* than the other pesticides used in this study. Etoxazole was the highest toxic against egg stage of two field populations of *T. urtica*e followed by clofentezine. Ochiai *et al.* (2007) studied the toxicity of the etoxazole and other pesticides against adult, larva and egg stages of *T. urticae.* Etoxazole showed no activity against adults of *T. urticae*. However, etoxazole was highly effective in controlling the larval and egg stages of *T. urticae* mite strain. The present results indicated that clofentezine was considerably toxic on the egg stage of two-spotted spider mite. The eggs treated with 29.7 ppm, 27.4 ppm in Baq'a and Deir-Alla field populations , respectively, both field populations had low resistance factor which mean that the clofentezine was still highly toxic against two spotted spider mite. Similar effect was recorded by Dingxu *et al.* (2006) for clofentezine and other pesticides against *Tetranychus* spp. The study of Dingxn *et al.*, (2006) indicated that clofentezine was highly toxic to different stage. The concentration required to kill 50% of the egg test population (LC_{50}) was 0.377 mg/l, while LC_{50}'s for larvae, protonymphs and deutonymphs were 20.747, 35.401 and 59.365 mg/l, respectively. Dingxu *et al.* (2006) showed that longevity of adult

females decreased significantly at LC_{50} dosages of clofentezine. Fecundity decreased significantly at LC_{50} when treated as larvae, and at LC_{25} and LC_{50} when treated as protonymphs. Similar results were obtained in the present study; clofentezine affected adult longevity and fecundity of all populations of *T. urticae* at LC_{50} dosages of clofentezine. Also clofentezine compound had effect on the hatchability of three populations of *T. urticae*. Historically, mites have developed resistance very quickly to ovicidal acaricides (Luis, *et al.,* 2008). Reports of resistance to clofentezine and cross resistance to hexythiazox in Australia after as few as four sprays of clofentezine were alarming. There were several fitness factors as increase fecundity, short egg stage, short development time for males and reduced adult longevity associated with the susceptible population which might have contributed to the development of resistance(Pree *et.al.,* 2002).Similar results were obtained in the present study in which reduced fecundity with reduced longevity significantly in the first and second generations of field populations compared with their control. But after second generations of field populations, longevity reduced continuously with increased fecundity significantly compared with first generations as response for clofentezine application .Different changes in the fitness factor do appear in the Syrian population after several treatments with clofentezine.

The adult fecundity and longevity decreased significantly in all generations of Syrian population. Resistant to clofentezine was detected in four populations of the European red mite, *Panonychus ulmi* (Tetranychidae: Acariformes*)* (Koch),which already resistant to hexythiazox and other compound in the apple orchards in Ontario after 5 years use with three to five clofentezine application (Pree *et. al.,*2002). Resistance to both clofentezine and hexythiazox was confirmed in *T. urticae* from apples and pears at Shepparton, Victoria after a total of 6-8 applications over 4-5 years (Edge, 1988).

Developed resistance under glasshouse condition in the two strain of *T. urticae*; first strain was sensitive for hexythiazox and clofentezine compounds ,second strain was resistant for hexythiazox but sensitive to clofentezine compound . Clofentezine resistance in first strain of the *T. urticae* was first confirmed after had been exposed to 40 applications of clofentezine over a 10 month period .But clofentezine resistance in *T. urticae* was detected after 5–6 sprays and caused field control failure (Sáenz-de-Cabezónand and Frank (2006). Resistance to clofentezine declined rapidly in a mixed population. Most resistance was lost in fewer than three generations under laboratory condition . However, mixed population under field condition lead to remove clofentezine resistance population during ten generations, at least for two seasons .There were several fitness factors (fewer eggs/female, longer egg stage, longer development time for males) associated with the resistant population which may have contributed to the loss of resistant phenotypes(Pree *et.al.,*2002).

In anticipation of wide spread use of clofentezine and hexythiazox by several researchers from Washington State University and Oregon State University began a joint project to investigate the management of mite susceptibility of *Panonychus. ulmi*, *T. urticae*, and *Tetranychus .mcdanieli* (Prostigmata: Tetranychidae) eggs to hexythiazox(Luis, *et.al.,* 2008) .The data suggested that population of the three major pest species of mite in the Pacific Northwest were susceptible to both hexythiazox and clofentezine (Luis, *et.al.,* 2008). Syrian population was observed highly susceptible to clofentezine by reduced fecundity and longevity during three generations .Syrian population that was treated with hexythiazox compound was increased fecundity significantly compared with the control. Hexythiazox resistance developed during three generation of susceptible strain of citrus red mite, but rapidly to a high level after 4^{th} generations. Resistance strain was 5-6 fold less

sensitive to the hexythiazox compared with susceptible strain because of the previous 18 treatment with the hexythiazox during six years in the field (Yamamoto *et. al.,* 1995). In the susceptible strain, the gene responsible for hexythiazox resistance might have accumulated in the selected population at the beginning of the hexythiazox treatment. However, at the 6^{th} generation an almost population with a high level of hexythiazox resistance could be established (Yamamoto *et. al.,* 1995). Mix mating between hexythiazox resistant strain and hexythiazox sensitive strain lead to the removal of the gene responsible for hexythiazox and then to the production of a strain more susceptible to hexythiazox. But the susceptibility increased slightly after five time of mating (Yamamoto *et. al.,* 1995). For managing hexythiazox resistance and keeping successful control of the species in the field, rotation and / or mixture programs seemed to be recommended as the practical tactics(Yamamoto *et. al.,* 1995).

Because etoxazole is a relatively new acaricide , studies on the etoxazole toxicity and its effect on the life biology of the TSSM could not be found . In the present toxicity test , it found that etoxazole has high toxicity to the spider mite egg. It's activity against egg hatchability was very high after etoxazole eggs direct treatment ; it is significantly reduced egg hatchability, but the fecundity and longevity of the adult that survived after ovicide treatment as egg stage were not significantly affected. Etoxazole is effective against eggs, larva and nymphs stage of mite, but lacks any efficacy against male and female. However, its sterilizing effect on the adult female by exhibits significant transovarial ovicidal activity was reported. However, several authors (Nauen and Smagghe, 2006).

Therefore, when applications of the etoxazole are made at recommended field rate, newly laid egg from treated females do not hatch. (Nauen and Smagghe, 2006, Janet ,2003) observed that ovicidal activity of etoxazole against egg stage of *Orius .insidiosus* (Hemiptera : Anthocoridae) eggs, seven days after treatment , *O. insidiosus* eggs hatching rate varied significantly among the acaricides tested. The hatching rate for the low (46%) and high rates (35%)were not significantly different between high and low rates, but were significantly lower than control , which had a hatching rate of 93% (Janet ,2003) **.** The chemically unrelated ovicide hexythiazox was released in 1987 in Australia and is also known to be very active on the eggs of *P. ulmi* in the laboratory (Welty *et al.,* 1988) and in the field (Bower, 1990). It was expected that clofentezine and hexythiazox would provide a period of excellent control of *P. ulmi,* during which time new acaricides would be developed. Several previous studies indicated that hexythiazox was a selective miticide active against various phytophagous mites of the agricultural importance and has no effect on natural enemies and beneficial insects (Janet, 2003). The compound has excellent ovicidal, larvicidal and nymphicidal actions. This study focuses on effects of Hexythiazox compound on the adult longevity, fertility and fecundity; fitness factors. The results indicated that females that survived treatment with hexythiazox as egg stage was found to have lower vitality with reduced hatchability significantly in Syrian population, and also has reducing effect on the adult longevity in the second and third generation compared with first generation in all population. In the two field populations hexythiazox has increased significantly effect on the egg hatchability during its application, respectively, for the three generations,but the egg hatching decreased significantly for each generation compared with their control. Results indicated that etoxazole and clofentazine compounds

had reduced significantly effect on the egg hatching for three generation of three different population compared with their control.

The widespread use of miticides for mite control has invariably lead to the development of resistance. During 1987, resistance was detected to the most recently registered chemicals, clofentezine and hexythiazox (Edge *et al.,* 1987). A lack of suitable replacements for these chemicals led to intensive study of various aspects of the resistance, including biological parameters. (Nadimi *et al,* 2008). They suggested a study of fitness parameters in a susceptible, resistant and hybrid (heterogeneous) strains which treated by hexythiazox. Mean total progeny production per female belong to the resistance mite strain was significantly higher than mean total progeny production per female belong to both strains susceptible, and hybrid (heterogeneous) strains which indicating greater fecundity.

Hexythiazox is an acaricide with a thiazolidinone structure. This compound is also not active on adults. However, eggs, larvae, protonymphs and deutonymph show an about equal susceptibility to hexythiazox. The effects of hexythiazox were assessed through following applications to different developmental stages of *Tetranychus urticae*. Recommended field rate of hexythiazox was used against adult. They had decreased longevity and net fecundity rate and causing over 90% mortality (Sekulic,1999).Generally speaking, spider mites have a history of rapidly developing resistance to miticides when a miticide is repeatedly applied to the same population. Alternating miticides that have different modes of action may reduce development of resistance to a specific miticide. In addition, other techniques to discourage resistance include spraying only when necessary. Etoxazole had no significant effect ($p>0.05$) on adult longevity compared to the first generation for all populations, but clofentezine had significant effect on the adult longevity

for the three generations of Syrian population, Baq’a and Deir-Alla field populations. However, clofentezine and etoxazole are selective ovicidal products. Spider mite eggs exposed to either compound fail to hatch as noticed. Both ovicides are selective and aid in the conservation of populations of beneficial arthropods. These products are typically used on several crops relatively early in the production season before mite populations reach outbreak conditions.

6. Conclusions and recommendations

6.1. Conclusions

The LC_{50}-values and the 95% confidence intervals were calculated from probit regressions using the SPSS13.0.0 computer program. Resistance factors (RF) were calculated by dividing the LC50-value of the field population by the LC_{50}-value of the Syrian population.LC_{50}- values of different acaricides were estimated by combining the results of three populations of *T. urticae*. This baseline LC_{50}- value served as a reference for the calculation of resistance factors for hexythiazox, etoxazole and clofentezine in *T. urticae* populations.

1. *T. urticae* field populations were more resistant against hexythiazox when compared with the etoxazole and clofentezine. Hexythiazox was the least toxic against egg stage of *T. urticae* than the other pesticides used in this study. Etoxazole was the highest toxic against egg stage of two field populations of *T. urtica*e followed by clofentezine.
2. Clofentezine was considerably toxic on the egg stage of two-spotted spider mite, both field populations had low resistance factor which mean that the clofentezine was still highly toxic against two spotted spider mite. But with repeated applications of the clofentezine for controlling field populations lead to develop resistance.
3. There were several fitness factors (increase fecundity, short egg stage, short development time for males and reduced adult longevity) associated with the susceptible population which may have contributed to develop resistance. The same result was observed in the several fitness factors; decrease longevity and increase fecundity in the two field populations.

4. Syrian population was highly susceptible to clofentezine and etoxazole . The effect of these compounds appeared as reduced fecundity during the three generations, also clofentezine had reduced effect on the adult longevity.
5. Females that survived treatment with hexythiazox as egg stage had lower vitality with reduced hatchability in Syria population, also had reduced effect on the adult longevity for second and third generations of all populations compared with the first generation.
6. In the two field populations treated with hexythiazox had a significant effect on the egg hatching for the three generations.
7. Etoxazole and clofentezine compounds had a significant decrease effect on the egg hatching for three generation of three different populations.
8. Etoxazole had high toxicity to the spider mite egg, since it has sterilizing effect on the adult female.
9. Etoxazole had no significant effect on adult longevity and adult fecundity compared to other acaricides
10. At recommended field rate hexythiazox was ineffective in controlling *T. urticae*. etoxazole , clofentezine were still effective to control *T. urticae.*

6.2. Recommendations

1. For managing hexythiazox resistance and keeping successful control of the species in the field, rotated programs is recommended.
2. Spider mites have a history of rapidly developing resistance to miticides when a miticide is repeatedly applied to the same population. Alternating miticides that have different modes of action might reduce development of resistance to a specific miticide.
3. Other techniques to avoid resistance include spraying only when necessary.
4. Clofentezine and etoxazole are selective ovicidal products. Spider mite eggs exposed to either compound fail to hatch as noticed. Both ovicides are selective and aid in the conservation of populations of beneficial arthropods.
5. Rotation and/ or mixture program using combination of the miticides/ insecticides , which show negative cross – resistance or delay resistance development is recommended.
6. When choosing an acaricide to treat a TSSM infestation, it is important to know how it will affect different life stages. It would be beneficial to have one acaricide that is effective against all life stages: eggs, larvae, nymphs, and adults.
7. If an acaricide is active against adult TSSM only, a second spray might be needed in a few days to control the newly hatched TSSM larvae. If an ovicide is used, the adult TSSM will lay more eggs, and thus a second spray might be needed.
8. A management strategy that uses two acaricides, which are toxic to different life stages, may extend the duration of TSSM control. For example, if etoxazole and clofentezine were used together, etoxazole would decrease the hatch rate of eggs that are already on the plants and Clofentezine would decrease the adult fecundity.

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8.Appendix

Table (12): Different concentrations of hexythiazox compound used to determent LC_{50} value against various populations of *T. urticae* data input.

Syrian population treated with hexythiazox	Total eggs	Response	Baq'a population treated with hexythiazox	Total eggs	Response	Deir-Alla population treated with hexythiazox	Total eggs	Response
0.02ppm	40	5	275ppm	40	7	200ppm	40	7
0.07ppm	40	13	300ppm	40	9	300ppm	40	17
0.09ppm	40	17	325ppm	40	16	400ppm	40	21
0.13ppm	40	25	350ppm	40	29	500ppm	40	25
0.18ppm	40	35	375ppm	40	35	600ppm	40	32

Table (13): Different concentrations of etoxazole compound used to determent LC50 vaule against various populations of *T. urticae* data input.

Syrian population treated with etoxazole	Total eggs	Response	Baq'a population treated with etoxazole	Total eggs	Response	Deir-Alla population treated with etoxazole	Total eggs	Response
0.02ppm	40	5	0.15ppm	40	6	0.05ppm	40	6
0.05ppm	40	11	0.30ppm	40	13	0.11ppm	40	15
0.09ppm	40	22	0.45ppm	40	19	0.17ppm	40	22
0.11ppm	40	25	0.60ppm	40	27	0.19ppm	40	25
0.15ppm	40	33	0.75ppm	40	34	0.28ppm	40	36

Table (14): Different concentrations of clofentezine compound used to determent LC50 value against various populations of *T. urticae* data input.

Syrian population treated with clofentezine	Total eggs	Response	Baq'a population treated with clofentezine	Total eggs	Response	Deir-Alla population treated with clofentezine	Total eggs	Response
5ppm	40	5	10ppm	40	6	5ppm	40	7
10ppm	40	13	20ppm	40	12	10ppm	40	14
15ppm	40	18	30ppm	40	20	15ppm	40	21
20ppm	40	24	40ppm	40	27	20ppm	40	27
25ppm	40	29	50ppm	40	32	25ppm	40	34

Table (15): Effect of the three acaricides on the fecundity of the two – spotted spider mite adults of the three populations after treatment with its LC_{50}, for three generations under laboratory condition data input.

Generation	Replicate	Syrian population treated with hexythiazox	Syrian population treated with etoxazole	Syrian population treated with clofentezine	Baq'a population treated with hexythiazox	Baq'a population treated with etoxazole	Baq'a population treated with clofentezine	Deir-Alla population treated with hexythiazox	Deir-Alla population treated with etoxazole	Deir-Alla population treated with clofentezine
G1	1	60	55	60	70	60	60	77	63	70
G1	2	64	60	55	78	67	68	73	60	68
G1	3	64	72	62	71	68	74	71	69	73
G1	4	68	57	54	80	61	63	79	55	79
G2	1	30	59	50	74	59	50	74	59	59
G2	2	29	63	48	71	60	52	75	66	52
G2	3	31	62	47	87	66	49	80	73	63
G2	4	33	57	49	86	70	51	86	68	51
G3	1	90	68	30	78	68	80	70	69	100
G3	2	79	62	30	83	69	81	74	72	120
G3	3	83	59	40	89	63	79	81	70	90
G3	4	95	66	41	79	60	78	89	66	95

G4	1	113	75	35	80	68	100	85	66	100
G4	2	99	53	38	77	71	150	90	71	137
G4	3	200	66	42	82	65	144	79	65	122
G4	4	100	78	37	76	69	98	77	78	200

Table (16): Effect of the three acaricides on the longevity of the two – spotted spider mite adults of the three populations after treatment with its LC_{50}, for three generations under laboratory conditions data input.

Generation	Replicate	Syrian population treated with hexythiazox	Syrian population treated with etoxazole	Syrian population treated with clofentezine	Baq'a population treated with hexythiazox	Baq'a population treated with etoxazole	Baq'a population treated with clofentezine	Deir-Alla population treated with hexythiazox	Deir-Alla population treated with etoxazole	Deir-Alla population treated with clofentezine
G1	1	33.9	33.8	31.8	34.2	34.3	32.5	33.8	34	32.7
G1	2	33.1	31.7	31.4	34	33.8	32.8	34	34.7	32.7
G1	3	34.6	32.4	33.1	33.6	33	30	33.2	32.1	33.2
G1	4	34.3	33	32	33	33.6	29.1	33.4	33.2	31.5
G2	1	33	33.4	24	33	34	28.7	33	32.2	27.6
G2	2	32.8	34.8	23.7	32.8	34.6	28	32.4	34	27.7
G2	3	33.3	33	23.9	32.6	33.9	26.2	33	32.7	27
G2	4	32.2	31.5	23	32.4	31.7	28.3	32.2	31.1	26.8
G3	1	33.7	32.2	16	33.7	33	13.2	32.9	33.7	14.9
G3	2	31.8	31.7	15.8	32.1	32.7	12.7	33.5	33.9	14
G3	3	32.4	32	16.4	31.9	32.2	13	32.5	33	15.1

تأثير ثلاثة مبيدات بيض على تكاثر سلالة حساسة و سلالتين حقليتين من الحلم الأحمر ذو البقعتين *(Tetranychus urticae* Koch (Acari: Tetranychidae**)** لثلاثة أجيال.

إعداد

اسراء وليد رجب سالم

المشرف

الأستاذ الدكتور توفيق مصطفى العنتري

الملخص

اجريت تجارب مخبريه لتقييم سمية ثلاث من المبيدات التقليدية و الحديثة شائعة الاستعمال على نبات الخيار في الأردن وهي: اتوكسازول و كلوفينتيزين وهكسي ثيازول ضد ثلاث مجتمعات من الحلم الأحمر ذو البقعتين (*Tetranychus urticae* Koch) جمعت من منطقتي البقعة و دير علا.وقد أحضرالمجتمع الثالث من مركز تربية الأعداء الحيوية من اللاذقية في سوريا و اعتبرت المجتمع الحساس .كان تركيز و كلوفينتيزين القاتل (ت ق.50 ،ت ق.90) لأعداد بيض الحلم للمجتمع السوري و المجتمعين المحليين: دير علا و البقعة على التوالي هو(16,56و 48,4)، (27,4 و 73,7)، (29,7 و 76,7) جزء من مليون و كان التركيز القاتل لمبيد وهكسي ثيازول (ت ق.50، ت ق.90) لأعداد بيض الحلم للمجتمع السوري و مجتمع ديرعلا ومجتمع البقعة على التوالي هو (0.10 و 0,24)، (370 و900) و(331 و386,17) جزء من مليون, و كان التركيز القاتل لمبيد اتوكسازول (ت ق.50، ت ق.90) لأعداد بيض مجتمعات الحلم، للمجتمع السوري والمجتمعين الحقليين دير علا و البقعة (0, 0 8 و 0.23) , (0,15 و 0,35) , (0,43 و 1,07) جزء من مليون و كانت النسبة ت ق.90 و التركيز الموصى به لمبيد كلوفينتيزين الحلم للمجتمع السوري و المجتمعين المحليين : دير علا و البقعة (0,322) , (0,491) ,(0,511) ضعفا على التوالي , بينما كانت النسبة بين ت ق .90 و التركيز الموصى به لمبيد وهكسي ثيازول للمجتمع السوري و المجتمعين المحليين : دير علا و البقعة (0,0048) , (7,72) ,(18) ضعفا على التوالي , بينما كانت النسبة بين ت ق .90 و التركيز الموصى به لمبيد اتوكسازول و هكسي ثيازوللمجتمع السوري و المجتمعين المحليين : دير علا و البقعة (0,0046) , (0,007) ,(0,021) ضعفا على التوالي .و بناءا على ذلك كان مبيد اتوكسازول هو الأكثر سمية كما أن هناك احتمال بفشل مبيد وهكسي ثيازول في المكافحة في حالت استعماله بالتركيز الموصى به حقليا. كما بينة النتائج أن مبيدي اتوكسازول و كلوفينتيزين يؤثران بشكل معنوي على نسبة التفقيس على مدى ثلاث أجيال لكل المجتمعات المستخدمة في هذه الدراسة مقارنة بالشاهد بينما يعمل مبيد وهكسي ثيازول يعمل على تقليل نسبة التفقيس في حالت المجتمع السوري كما تقل نسبة التفقيس في حالت المجتمعين المحليين مقارنة بالشاهد .

كما بينت النتائج أن مبيد اتوكسازول لا يوجد له أي تأثير معنوي في عمر الأنثى ولا على عدد البيض الذي تضعه الأنثى الواحدة خلال فترت حياتها. بينما يؤثر مبيد كلوفينتيزين بشكل ملحوظ في فتره حياة الطور الكامل للأنثى على مدى ثلاث أجيال في كل المجتمعات المستخدمة في هذه الدراسة, أما بالنسبة لتأثير هذا المبيد على عدد البيض الموضوع من الأنثى الواحدة خلال فترة حياتها فقد اعتمد على المجتمع الذي تنتمي له الأنثى , حيث لوحظ انه يقلل من عدد البيض الذي تضعه الأنثى التي تنتمي للمجتمع السوري على مدى ثلاث أجيال بشكل ملحوظ , بينما يبدأ عدد البيض الموضوع من الأنثى الواحدة التي تنتمي لأحد المجتمعين المحليين بالتزايد بشكل ملحوظ ما بعد الجيل الثاني. في حالت معاملة المجتمعات بمبيد وهكسي- ثيازول فقد أظهرت النتائج أن تأثير هذا المبيد يعتمد على المجتمع المعامل ففي حالت معاملة المجتمعين المحليين فان هذا المبيد لم يظهر أي تأثير على عدد البيض الموضوع من الأنثى المنتمية لهذين المجتمعين. بينما كان تأثيره واضحا في حالت المجتمع السوري ما بعد الجيل الثاني حيث ازداد عدد البيض الموضوع من الأنثى المنتمية لهذا المجتمع بشكل ملحوظ . كما بينت النتائج أن تأثير وهكسي ثيازول في فترة حياة الأنثى ذات الطور الكامل يعتمد على المجتمع المعامل بهذا المبيد .